OPEN ACCESS

IJABR

International Journal of Advanced Biotechnology and Research (IJABR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-15, Issue1, 2024, pp41-47
DOI: 10.5281/zenodo.10823919

Research Article

Different approaches for improvement of acclimatization step of in vitro micropropagated local Juglansregia L. genotypes in Uzbekistan

Guljakhon Eshbekova [1], Bakhtiyor Kodirov[2] and Zafar Ismailov[1]

Article Info
[1]Molecular Biotechnology Lab, Department of Genetics and Biotechnology, Samarkand State University named after Sharof Rashidov, University Boulevard 15, Samarkand, 140100, Uzbekistan
[2]In vitro laboratory of SAG-Agro "Bog'bon", Samarkand, 140100 Uzbekistan
----
*Correspondence: guljakhonbio@mail.ru

Received: 14/02/2024
Accepted: 11/03/2024
Published: 16/03/2024

Abstract.
Tissue culture of Persian walnut (Juglansregia L.) is considered difficult compare to other crops. In this research, different methods, including seed starters and polystyrene cups, as well as pot mixture in different ratiowere used to increase the efficiency of the acclimatization stage after microclonal propagation of local varieties and forms. According to the results of the research, the survival rate in all genotypes: Ideal variety, PDM23 form and 202YaKT form was higher, respectively, 58.3%, 65.8%, 29.2 % when seed starters or polystyrene cups were used in the peat: biohumus: perlite mixture in the ratio of 1:2:1. Also, the success of the acclimatization stage depends on the genotype, and the 202YaKT form showed a low performance compared to other genotypes.

Keywords: Juglansregia L., microclonal propagation, acclimatization, local variety, Ideal variety, PDM23 form, 202YaKT form.

Introduction.

Today, one of the best methods for mass propagation of walnut (Juglansregia L.) in order to establish orchards on an industrial scale is in vitro microclonal propagation [1,2,3]. However, the rooting stage of that process is consideredmore difficult; the acclimatization stage also has some limitations. The results of the research showed that the quality of invitro micro shoots, formation of roots, the characteristics of stomata on the leaves, the presence of sufficiently healthy leaves, and the genotype are important factors for the successful acclimatization stage [4,5,6].

Cite This article: Guljakhon Eshbekova, Bakhtiyor Kodirov and Zafar Ismailov, 2024. Different approaches for improvement of acclimatization step of in vitro micropropagated local Juglansregia L. genotypes in Uzbekistan. International Journal of Advanced Biotechnology and Research (IJABR) ISSN 0976-2612, Online ISSN 2278–599X, Vol-15, Issue1, 2024, pp41-47 DOI: 10.5281/zenodo.10823919

Low water capacity in the multiplication and rooting stages of in vitro explants also affected the survival rate in the next adaptation stage [6].In addition, the substrate used for acclimatization and adherence to the standard rules of temperature and humidity during the process are considered to be the most important rules [7]. Several studies have been conducted to increase the efficiency of the acclimatization stage, mainly related to changing the temperature and humidity for different periods of time. At the initial stage, direct sunlight and low temperature (<16 ℃) in the growth chamber caused a decrease in survival rate [8,9].

After the in vitro explants transferred to the ex-vitro stage, it was observed that the relative humidity was higher than 80-90% during the first two weeks, and when it was gradually reduced to 60% during the next 2-3 weeks, the condition of the leaves was well preserved in the seedlings [10]. When peat, perlite and vermiculite in 1:1:1 ratio [11] and peat and vermiculite in 1:1 ratio [12] were used as substrate, 60.5% and 79.2% survival was observed respectively. In the course of research, it was found that the result of microclonal propagation of walnut, as well as the stage of acclimatization, depends on the genotype [13]. However, insufficient studies have been carried out on the composition and ratio of the substrate during the acclimatization stage. In this study, the results of the use of different substrates for the acclimatization of local walnut genotypes of Uzbekistan and the use of different methods to maintain high relative humidity for seedlings in the first two weeks are presented.

Materials and methods.

Plant Materials

Three Persian walnut (J. regiaL.) genotypes were obtained from Scientific-research institute of Horticulture, Viticulture and Wine-making (Uzbekistan): variety ‘Ideal’ (N41°36.157; E70°05.767) and ‘Form 202YaKT’ (N41°36.157; E70°05,767), ‘Form PDM23’ (N39°73.852; E66°73,381)[14,15]. Initial explants were taken from stem cuttings multiplied in vitro for at least 10 subcultures.

Multiplication.

Uninodal explants were cultured in Driver and Kuniyuki medium(DKW) [10] with 96 mg/ L^{-1} Fe-EDDHA, supplemented with1.5 mg/L^{-1} BAP, 0.01 mg/L^{-1} IBA, 30 g/L^{-1} sucrose, 0.4mM phloroglucinol and gelled with 5.5 g/L^{-1} Agar. The pH was adjusted to 5.7 before autoclaving.

Root induction.

Explants of three genotypes collected during the multiplication phase were cultured in DKW medium, with 1 /2 macronutrients and 96 mg/L^{-1} Fe-EDDHA, added with 3 mg/L^{-1} IBA, 30 g L^{-1} sucrose, gelled with 5.5 g/L^{-1} Agar. The pH was adjusted to 5.7 before autoclaving.

Acclimatization.

To increase the efficiency of the acclimatization step, invitro micropropagated explants were adapted to greenhouse conditions in 3 different ways:

1. Initially, rooted microshoots were planted in peat tablets (41 mm diameter) on open nursery trays and incubated in mini-greenhouses under controlled conditions (Fig.1 B,E). Mini greenhouses were covered with glass to maintain high relative humidity (90±2%). After the 3rd week, glass covers were removed slowly, in such a way that plants were acclimated step-by-step to a gradually-reduced humidity. In this stage a photoperiod of 16/8 h was used, with an average light intensity of 3500 lx; whereas temperature was 24–26 ℃. During the 4th week, surviving plants were transplanted to plastic cups (500 cm^3, upper diameter 93 mm, lower diameter 55 mm, height 135 mm) with a two types of mixture (rate 1:1:1,1:2:1) of peat, biohumus and perlite. Pots were placed in a greenhouse under normal conditions,

with oscillating temperatures and relative humidity ranging from 17 to 32 °Cand from 45 to 75%, respectively, and an average maximum light intensity of 4600 lx. Plants were watered 1 to 2 times per week, depending on the weather and the state of the substrate. When micropropagated plants were acclimatized (6th week), survival and quality of plants of each genotype from both culture media were counted, and the general state was annotated.

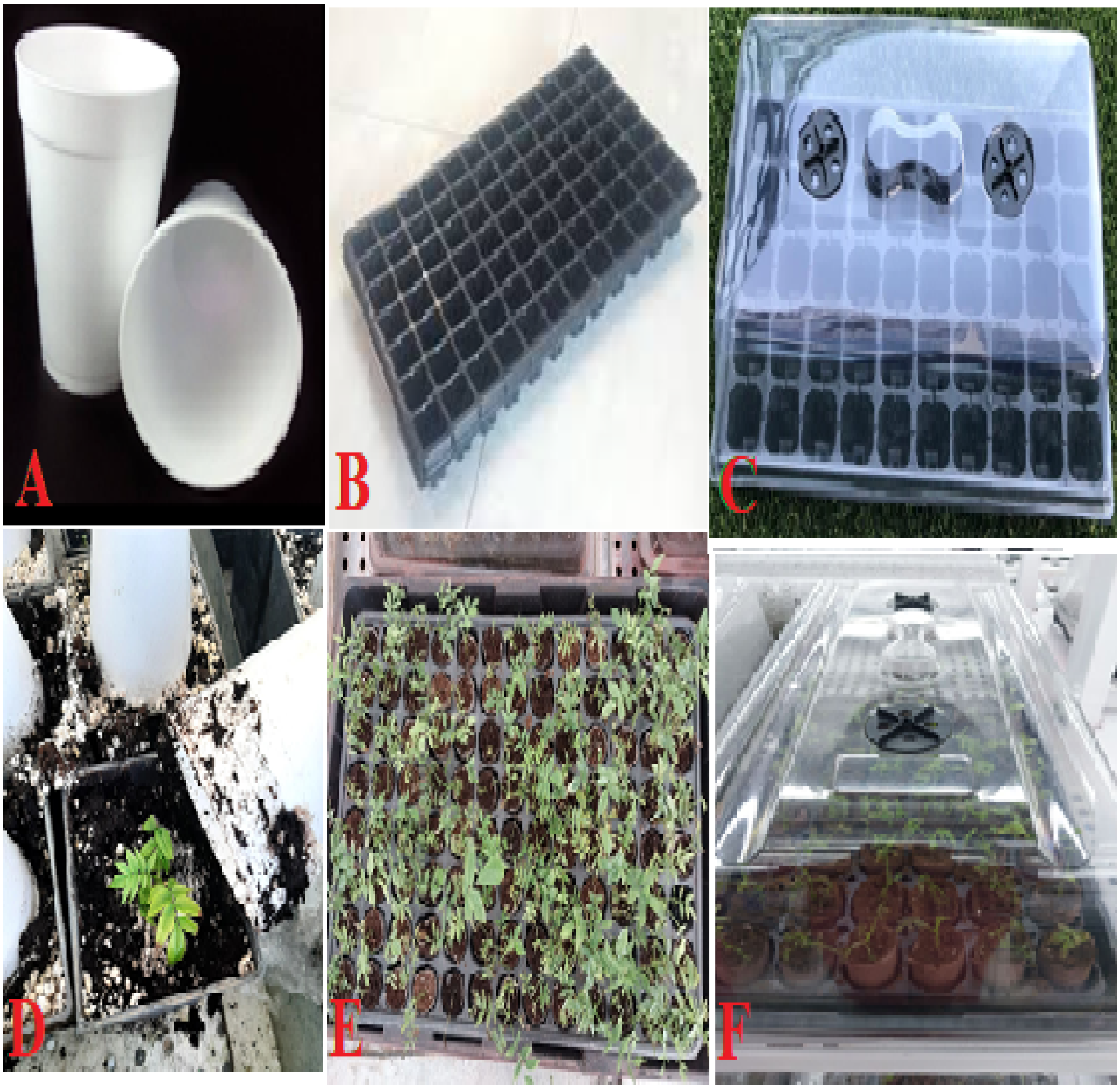

Figure 1.A,B,C-polysterine cups, open nursery trays, seed starters used in experiments; D,E,F-Seedlings during acclimatization (initial two weeks) under the polysterine cup, on open nursery trays, inside of seed starters.

2. Rooted microshoots were transferred directly to pots containing two different mixtures (500 cm^3, upper diameter 93 mm, lower diameter 55 mm, height 135 mm) and covered with polystyrene cups (Fig.1 A,D). Through this, 95 ± 2% humidity was provided for seedlings. In the first 2 weeks, the cups were not opened in order to ensure high humidity (95 ± 2%) for the plants under the polystyrene cups. Then, in the 3rd week, holes were opened in the upper part of the polystyrene cups, and in the 4th week, the polystyrene cups were removed. Then, pots

were placed in a greenhouse under normal conditions.

3. Rooted microshoots were planted in vitro peat tablets (41 mm diameter) and incubated in seed starter containers (Fig.1 C,F). In this case, the valves located in the lids were not opened in order to ensure high humidity (95 ± 2%) in the first 2 weeks. Then, in the 3rd week, the valves were opened, and in the 4th week, the transparent lids were opened. Then, pots were placed in a greenhouse under normal conditions.

Statistical analysis.

Statistical analysis was performed using Origin 8.6 software (Microcal Software Inc., Northampton, MA). Results were expressed as mean ± SE. All experiments were repeated three times and 120 plants were treated in each experiment.

Results and discussion.

Some authors reported that biohumus can be used instead of peat when growing plants in greenhouse conditions [16,17]. By adding biohumus to the pot mixture, seedlings can be fed in sufficient quantity.

Table 1. Survival rate of seedlings on different methods and ratio of potting mixture during acclimatization step.

Methods for improving efficiency of acclimatization	Survival rate of seedlings (%)					
	Ideal variety		PDM23 form		202YaKT form	
	1:1:1	1:2:1	1:1:1	1:2:1	1:1:1	1:2:1
Seed starters	46.7±2.5	54.2±2.6	51.7±2.4	65.8±2.3	19.1±0.8	29.2±1.8
Polysterine cups	48.3±1.9	58.3±2.8	56.7±3.1	60±1.8	NE	NE
Open nursery trays	25.8±1.2	31.7±1.6	22.5±1.3	37.5±1.1	NE	NE

Values in each column represent means ± SE.NE-non evaluated.

Also, it allows reducing the amount of greenhouse gas released when peat decomposes [18,19]. The results of the study showed that the survival rate of all three genotypes: Ideal variety, PDM23 form and 202YaKT form showed a high result in peat: biohumus: perlite mixture in the ratio 1:2:1 (58.3%, 65.8%, 29.2%, respectively).

It was found that the genotype is important in the microclonal propagation of walnut, as well as in the acclimatization stage [20,21]. Two of the 3 genotypes selected in this study, Ideal variety and form PDM23, showed successful performance at all stages, however, rooting and acclimatization index were relatively low in form 202YaKT (the highest acclimatization index was 29.2%).

In the first 2-3 weeks during the acclimatization stage, maintaining high humidity and protecting seedlings from various external environmental influences are important for the successful process [22,23]. For this reason, in order to maintain high humidity and increase the survival rate in the first weeks, seed starter containers, polystyrene cups were used and compared with seedlings planted in open nursery trays. There were significant differences on the survival rate of plantlets which acclimatized on open nursery trays and seedling starters, as well as under polystyrene cups. The survival rate of seedlings of the Ideal variety was 31.7% when acclimatized in nursery trays with 1:2:1 peat: biohumus: perlite mixture, and 58.3% when covered with polystyrene cups. The survival rate of PDM23 form seedlings acclimatized in nursery trays was 37.5% in 1:2:1 peat: biohumus: perlite mixture, and 65.8% in seedling starter. Therefore, the use of different methods to ensure high humidity for seedlings during the acclimatization of seedlings propagated by the microclonal method may allow to increase the survival rate.

Conclusion.

To conclude, it is possible to use biohumus in the pot mixture to increase the efficiency of acclimatization stage of walnut microclonal propagation. It is also effective to use different methods, including seed starters or polystyrene cups, to ensure high humidity for seedlings during the first two weeks. Furthermore, the efficiency of the acclimatization stage highly depends on the genotype.

Funding: None

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: None

Conflicts of Interest: The authors declare no conflict of interest.

References:

1. Vahdati, K.,Sadeghi-Majd, R.,Sestras, A.F.,Licea-Moreno, R.J.,Peixe, A. andSestras, R.E., (2002),“Clonal Propagation of Walnuts (Juglansspp.): A Review on Evolution from Traditional Techniques to Application of Biotechnology,”Plants,11(22), pg. 3040. https://doi.org/10.3390/plants11223040
2. Ribeiro, H.,Ribeiro, A., Pires, R., Cruz, J., Cardoso, H.,Barroso, J.M., andPeixe, A., (2022),“Ex Vitro Rooting and Simultaneous Micrografting of the Walnut Hybrid Rootstock ‘Paradox’ (Juglanshindsi ×Juglansregia) cl. ‘Vlach’,”Agronomy,12(3), pg. 595.https://doi.org/10.3390/agronomy12030595
3. Zarghami, R. andSalari, A., (2015),“Effectof different hormonal treatments onproliferation and rootingof three Persian walnut (JuglansregiaL.) genotypes,”Pak. J. Biol. Sci.,18(6),pg. 260–266.https://doi.org/10.3923/pjbs.2015.260.266
4. Vahdati, K., Leslie, C.,Zamani, Z. andMcGranahan, G., (2004),“Rooting and acclimatization of in vitro-grown shoots from mature trees ofthree Persian walnut cultivars,”HortScience,39(2), pg. 324–327. http://dx.doi.org/10.21273/HORTSCI.39.2.324
5. MalekiAsayesh, Z.,Vahdati, K.,Aliniaeifard, S. andAskari, N., (2017),“Enhancement of ex vitroacclimatization of walnut plantlets throughmodification of stomatal characteristics in vitro,”Sci. Hortic.,220(16), pg. 114–121.https://doi.org/10.1016/j.scienta.2017.03.045
6. Asayesh, Z.M.,Vahdati, K. andAliniaeifard, S., (2017),“Investigation of physiological components involved in low water conservationcapacity of in vitro walnut plants,” Sci. Hortic.,224(20),pg. 1–7.https://doi.org/10.1016/j.scienta.2017.04.023
7. Licea-Moreno R.J., Contreras A., Morales A.V., Urban I., Daquinta M. andGomez L., (2015),“Improvedwalnut mass micropropagationthrough the combined use ofphloroglucinol and

FeEDDHA,” PCTOC, 123, pg. 143–154. http://dx.doi.org/10.1007/s11240-015-0822-3

8. Navatel, J.C.; Bourrain, L., (2001), Plant production of walnut JuglansregiaL. by in vitro multiplication. ActaHortic. 544, pg. 465–471.
9. Hassankhah, A., Vahdati, K., Lotfi, M., Mirmasoumi, M., Preece, J. and Assareh, M. H., (2014), “Effects of ventilation and sucrose concentrations on the growth and plantlet anatomy of micropropagated Persian walnut plants,” Int. J. Hortic. Sci. Technol., 1(2),pg. 111–120. https://doi.org/10.22059/ijhst.2014.52781
10. McGranahan, G. H., Driver, J.A., Tulecke, W., (1987), “Tissue culture of Juglans,”Cell and Tissue Culture in Forestry, 24(26), pg. 261–271. https://doi.org/10.1007/978-94-017-0992-7_19
11. Stevens, M.E. andPijut, P.M.,(2018), “Rapid in vitroshootmultiplicationof the recalcitrant species JuglansnigraL,”In Vitro Cell. Dev.Biol.-Plant,54(3),pg. 309-317. https://doi.org/10.1007/s11627-018-9892-3
12. Yegizbayeva, T.K., Garcia, G.S., Yausheva, T.V., Kairova, M., Apushev, A.K., Oleichenko, S.N. andLicea-Moreno, R.J., (2021),“Unraveling Factors Affecting MicropropagationofFour Persian Walnut Varieties,”Agronomy,11(7), pg. 1417. https://doi.org/10.3390/agronomy11071417
13. Chenevard, D., Jay-Allemand, C.,Gendraud, M. andFrossard, J.,(1995), “The effect of sucrose on thedevelopment of hybrid walnutmicrocuttings (Juglansnigra × Juglansregia). Consequences on their survival during acclimatization,”Ann. For. Sci.,52(2), pg. 147-156. https://doi.org/10.1051/forest:19950205
14. Butkov, E.A., Mamutov,B.Kh.,Nikolyai, L.V. andKasimkhodjaev, A.,(2020), “Study on the selectionof the best formsof walnut in Uzbekistan,” IOP Conference Series: Earth and Environmental Science, Tashkent, Uzbekistan, 614(1). https://doi.org/10.1088/1755-1315/614/1/012107
15. Eshbekova G.G., Kodirov B.E.,Ismailov Z.F., (2023), Selection of local walnut varieties and forms for microclonal propagation and preparation of introduction material, Food security: national and global problems. 4, pg.21-25.
16. Bachman, G. R., & Metzger, J. D. (2008). Growth of bedding plants in commercial potting substrate amendedwith vermicompost. Bioresource Technology, 99(8), pg.3155-3161. http://dx.doi.org/10.1016/j.biortech.2007.05.069
17. Zaller, J. G. (2007). Vermicompost as a substitute for peat in potting media: Effects on germination, biomassallocation, yields and fruit quality of three tomato varieties. ScientiaHorticulturae, 112, pg.191-199.http://dx.doi.org/10.1016/j.scienta.2006.12.023
18. J. Theunissen, P. A. Ndakidemi, C.P. Laubscher, (2010), “Potential of vermicompost produced from plant waste on the growth and nutrient status in vegetable production”, International Journal of the Physical Sciences, vol. 5, issue 13, pg. 1964-1973.
19. G. V. Prasanna Kumar, H. Raheman,(2012), “Identification of optimum combination of proportion of vermicompost in the soil-based potting mix and pot volume for the production of paper pot seedlings of vegetables”, Journal of plant nutrition, vol. 35, pg. 1277-1289.

20. Cheniany, M.; Ebrahimzadeh, H.; Masoudi-Nejad, A.; Vahdati, K.; Leslie, C.(2010), Effect of endogenous phenols and some antioxidantenzyme activities on rooting of Persian walnut (JuglansregiaL.). Afr. J. Plant Sci. 4, pg.479–487.
21. Cheniany, M.; Ebrahimzadeh, H.; Masoudi-Nejad, A.(2012),Expression of chalcone synthase influences flavonoid content and frequencyof rhizogenesis in microshoots of JuglansregiaL. Plant Cell Tissue Organ Cult. 109, pg. 51–59.
22. Gotea, R.; Gotea, I.; Sestras, R.E.; Vahdati, K. (2012), In vitro propagation of several walnut cultivars. Bull. Univ. Agri. Sci. Vet. Med.Cluj-Napoca. Hortic. 69, pg. 167–171.
23. Leal, D.R.; Sánchez-Olate, M.; Avilés, F.; Materan, M.E.; Uribe, M.; Hasbún, R.; Rodríguez, R. (2007). Micropropagation of JuglansregiaL.In Protocols for Micropropagation of Woody Trees and Fruits; Jain, S.M., Häggman, H., Eds.; Springer: Dordrecht, The Netherlands, pg. 381–390.